contents of the flask was transferred to 100 ml of tryptic soy broth (TSB) containing 25 μg of labeled methylmercury bromide, [^{203}Hg]CH_3HgBr (MMB), with a radioactivity of 1.71×10^6 disintegrations per minute. Figure 2 depicts the evolution of Hg^0 during the growth of the mixed culture as compared to that of an uninoculated control containing MMB. Analysis of the contents of the flask after 170 hours of incubation indicated the complete disappearance of methylmercury. (The methylmercury was completely recovered from the control flask.) The discrepancy arising from the fact that only 42 percent of the methylmercury added was recovered (as Hg^0) although all the methylmercury had disappeared from the flask contents may be explained in terms of the radioactivity present in the cell fraction (*6, 7*).

Serial dilutions of the mixed culture from the TSB flask were plated out on tryptic soy agar, and representative colonies were selected from high-dilution plates. After the cultures had been purified by streaking, they were tested for methylmercury degradation by incubation in TSB containing 0.25 part per million (ppm) of labeled MMB (radioactivity, 1.03×10^6 disintegrations per minute). Four of these cultures degraded methylmercury, as shown in Fig. 3. Although these species have not been characterized, all were short, Gram-negative rods and appear to be *Pseudomonas* sp.

The pseudomonads isolated appear to be quite similar to the species isolated from soil by Tonomura *et al.* (*7*) and reported to convert organomercurials to metallic mercury and an organic moiety. More recently, the enzyme system involved has been isolated (*8*) and characterized by Japanese workers (*9*).

The degradation of methylmercury was further verified by short-term incubation of cultures that degraded methylmercury with 25 μg of MMB in closed flasks containing 50 ml of TSB medium. The head space gases were analyzed for methane by flame-ionization gas chromatography with an OV-1 column. Methane was produced by the four cultures with concomitant volatilization of mercury, an indication that the methylmercury was degraded to methane and Hg^0. No methane was produced by cultures that did not degrade methylmercury incubated under the same conditions.

The presence of Hg^0 in the head space gas of one of the isolates (sample 1-2) was confirmed by mass spectrometry. Head space gases from an inoculated control without methylmercury and an uninoculated control containing 5 ppm of MMB did not contain Hg^0.

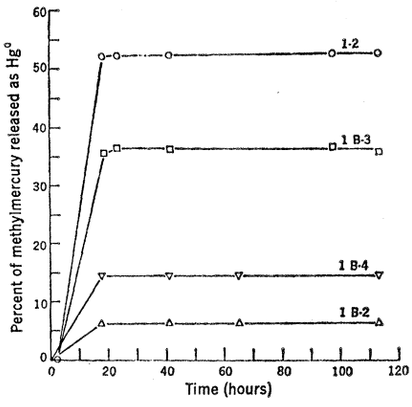

Fig. 3. Degradation of methylmercury by pure cultures isolated from sediment. Volatile mercury was monitored in a trap containing $HgBr_2$ and KBr attached to the flask. Curves are shown only for cultures that degraded methylmercury.

The results indicate that demethylators are present in mercury-containing sediments and could be responsible for the degradation of at least some of the methylmercury produced in sediments. Thus these organisms may serve a useful purpose in maintaining the environmental methylmercury concentrations at a minimum. However, data are not available in the literature on the prevalence of such organisms in the aquatic environment or the conditions which might favor their growth. We have isolated some 200 cultures from various sediments and environmental samples taken from areas where fish containing methylmercury have been found. The results of experiments, currently in progress, with these isolates will be presented elsewhere (*10*).

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4. We confirmed the quantitative trapping of DMM and Hg^0 by preliminary experiments, using $HgCl_2$, CH_3HgBr, and Hg^0 (produced in aqueous solution by the chemical reduction of Hg^{2+}), all labeled with ^{203}Hg, and unlabeled DMM. Of the labeled mercury species tested, only Hg^0 and DMM were found in the $HgBr_2$/KBr trap. If DMM were formed from [^{203}Hg]-MMB by a microbial reaction in the incubation flask, the [^{203}Hg]DMM formed would volatilize to the trap and be converted to [^{203}Hg]-MMB in the trapping solution. The resulting radioactivity would be seen as a false positive test for Hg^0. To eliminate this possibility, all trap contents containing radioactivity were extracted and analyzed for methylmercury by electron-capture gas chromatography.
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6. The residual radioactivity in the flask contents is thus assumed to be attributable to Hg^0 bound to cell surfaces.
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Natural Abundance Carbon-13 Nuclear Magnetic Resonance Spectra of Human Serum Lipoproteins

Abstract. *Human serum lipoproteins have been studied by Fourier transform nuclear magnetic resonance of carbon-13 in natural abundance. Spectra of high-density, low-density, and very-low-density lipoproteins were recorded and partly assigned. The prominent features of these spectra reflect the qualitative and quantitative composition of the lipid moiety of these complexes. The results suggest that carbon-13 nuclear magnetic resonance will be a useful technique for studies of the structural and dynamic parameters of lipoproteins.*

The structure and metabolism of human serum lipoproteins have come under increasing scrutiny in recent years, reflecting accentuated interest in the topic of protein-lipid interactions generally and the recognition of the important physiological roles of lipoproteins specifically (*1*). The structural properties of serum lipoproteins have been probed by a variety of physical and chemical techniques; nevertheless, the structural organization of these

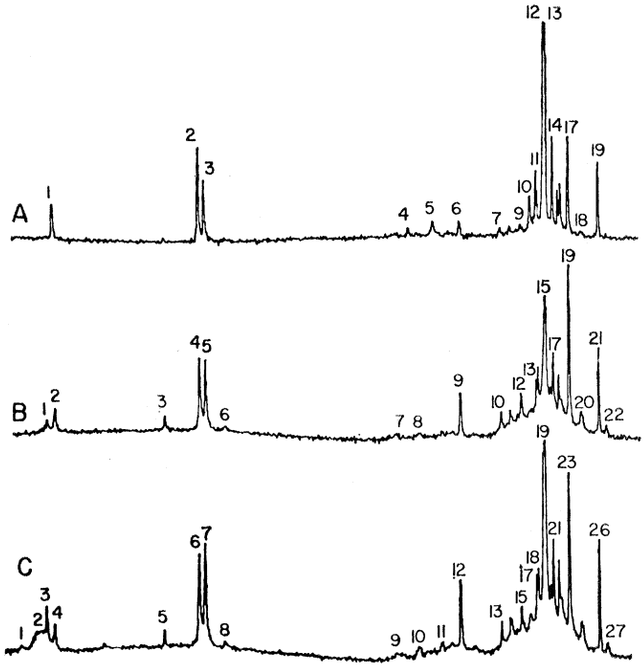

Fig. 1. Natural abundance ^{13}C Fourier transform NMR spectra of aqueous human serum lipoproteins at 15.18 Mhz. Spectra were recorded under conditions of proton decoupling in spinning 20-mm sample tubes, at 40°C with a recycle time of 3 seconds. Chemical shifts are given in Table 1. (A) VLDL, 80 mg/ml, after 8,192 accumulations; (B) LDL, 110 mg/ml, after 16,384 accumulations; and (C) HDL, 125 mg/ml, after 16,384 accumulations.

Table 1. Chemical shifts and assignments of the ^{13}C resonances of human serum lipoproteins. The abbreviations VLDL, LDL, and HDL stand for very-low-density, low-density, and high-density lipoproteins, respectively. Chemical shifts are in parts per million (ppm) upfield from CS_2. The terminal methyl group of fatty acyl chains at 178.5 ppm upfield from CS_2 (*11*) was used as the internal reference. Unless otherwise noted, the assignments refer to the fatty acyl chain. The numbers in parentheses after the shifts for VLDL, LDL, and HDL designate the peaks in Fig. 1, A, B, and C, respectively. Model values are chemical shifts in a model compound; Glu, glutamic acid; **C**, the specific carbon the assignment is for.

Chemical shift (ppm)				Assignment
VLDL	LDL	HDL	Model	
		11.6(1)	11.3*	Glu carboxyl
		~18 (2)	15–23†	Protein carbonyl
	18.8(1)	18.8(3)		Carbonyl‡
20.6(1)	21.1(2)	21.1(4)	19.4§, 19.7§	Carbonyl
	52.7(3)	52.7(5)	52.6‖	Cholesterol C5
62.8(2)	62.8(4)	62.8(6)	62.5¶, 62.3§	$-CH=\mathbf{C}H-CH_2-CH_2-$
64.5(3)	64.5(5)	64.5(7)	64.5§	$-CH=\mathbf{C}H-CH_2-CH=CH-$
	70.2(6)	70.0(8)	69.9‖	Cholesterol C6
	119.7(7)	119.7(9)	118.8‖	Cholesterol C3
123.4(4)			123.5§¶	Glycerol CH
	126.5(8)	126.2(10)	126.4#	Choline CH_2-N
130.6(5)			130.4§¶	Glycerol CH_2
		132.9(11)	133.0#	Choline CH_2-O
138.3(6)	138.4(9)	138.3(12)	138.4#	Choline $(CH_3)_3N$
150.2(7)	150.2(10)	150.2(13)	150.0‖	Cholesterol C13
152.9(8)	152.6(11)	153.0(14)	152.7‖	Cholesterol C24**
155.9(9)	156.0(12)	156.0(15)	155.8‖	Cholesterol C10
158.7(10)		158.5(16)	158.5§¶	CH_2-CH_2-CO-O
160.5(11)	160.5(13)	160.4(17)	160.6¶	$CH_3-CH_2-CH_2-CH_2$
160.9††	160.9(14)	160.9(18)	161.0§	
162.7(12)	162.7‡‡	162.7‡‡	162.7–163.5§¶	Fatty acyl $(CH_2)_n$
163.1(13)	163.1(15)	163.1(19)		
	164.6(16)	164.6(20)	164.3‖	Cholesterol C25
165.3(14)	165.3(17)	165.3(21)	165.3§¶	$-CH_2-CH_2-CH=CH-$
166.9(15)	166.9(18)	166.9(22)	166.8§	$-CH=CH-CH_2-CH=CH-$
167.6(16)	167.3‡‡	167.3‡‡	167.6¶, 167.7§	$-CH_2-CH_2-CO-$
169.8(17)	169.8(19)	169.8(23)	169.8¶, 169.9§ 169.8‖, 169.6‖	$CH_3-CH_2-CH_2$ Cholesterol C26, C27
§§	173.1(20)	173.1(24)	173.2‖	Cholesterol C19
§§	174‡‡	174.0(25)	173.6‖	Cholesterol C21
178.5(19)	178.5(21)	178.5(26)	178.5§	CH_3-CH_2
	180.7(22)	180.7(27)	180.5‖	Cholesterol C18

* Glutamic acid, from Horsley *et al.* (*15*). The α-carbon chemical shift of glycine was taken at 151.1 ppm upfield from CS_2. † Aqueous proteins (*4*). ‡ Cholesteryl fatty-acyl ester, tentative assignment. § Trilinolein in chloroform (*11*). ‖ Cholesteryl acetate in dioxane-chloroform (*9*). ¶ Triolein in chloroform (*11*). # Dipalmitoyl lecithin in D_2O (*10*). ** May also contain ε carbon of lysine and β carbon of leucine in the spectrum of HDL. †† Peak slightly upfield from peak 11 in Fig. 1A. ‡‡ Shoulder. §§ Peak in Fig. 1A, at about 173 ppm.

complexes and the nature of the lipid-protein interactions responsible for it remain uncertain. Preliminary efforts to study serum lipoproteins by employing proton magnetic resonance spectroscopy (*2*) have yielded rather little insight into these matters but do suggest that nuclear magnetic resonance (NMR) techniques have the potential of supplying important information.

Relative to proton NMR, ^{13}C NMR spectroscopy offers definite advantages for the study of structure at the level of complexity of lipoproteins. These include (i) increased resolution, (ii) more readily assigned resonances when complete proton decoupling is employed, and (iii) relative ease of obtaining information concerning the segmental mobility of the molecules involved (*3*). Inherent disadvantages of ^{13}C NMR, namely poor sensitivity and low natural abundance of the ^{13}C nucleus, have been partially overcome by the development of pulsed Fourier transform NMR instruments, the use of large sample tubes, and the digital accumulation of many individual scans. Nevertheless, systems of high molecular weight and significant structural complexity must be studied at rather high concentrations to obtain ^{13}C NMR spectra of good quality in reasonable lengths of time (*4*). The recent development of a 20-mm probe (*5*) has significantly alleviated these disadvantages by providing a threefold increase in sensitivity compared to conventional 12-mm and 13-mm probes and has prompted the examination of serum lipoproteins by employing ^{13}C NMR. Results of our preliminary studies are reported here.

The NMR spectrometer consists mainly of a "home-built" pulsed NMR apparatus built around a Varian 14.2-kgauss electromagnet, corresponding to a ^{13}C frequency of 15.18 Mhz; a Fabri-Tek 1074 signal averager; and a Digital Equipment Corporation PDP-8/E computer. The probe uses spinning sample tubes of 20-mm outside diameter (*5*).

Lipoproteins were prepared from freshly collected serum from healthy adults by the method of ultracentrifugal flotation in solutions of varying concentrations of KBr containing about $5 \times 10^{-4} M$ ethylenediaminetetraacetic acid (*6*). Lipoproteins were isolated and purified in the following density ranges: very-low-density lipoproteins (VLDL), 0.95 to 1.019; low-density lipoproteins (LDL), 1.019 to 1.063; and high-density lipoproteins (HDL), 1.063 to 1.21. The purity of the lipoproteins was examined

by electrophoresis on 2 percent agarose gels in a tris(hydroxymethyl)aminomethane-glycine buffer, *p*H 8.6; in each case, only a single band was observed. Protein determinations were made by the method of Lowry *et al.* (*7*) and the results were employed to calculate lipoprotein concentrations, by using the known protein content of each lipoprotein class (*1, 8*). Samples were prepared for NMR study by concentration of the isolated lipoproteins in an Amicon cell with a PM 30 membrane, followed by exhaustive dialysis against 0.01*M* phosphate buffer at *p*H 7.4. To retard deterioration of the samples, they were layered with argon, and 1 mg of sodium ampicillin was added per milliliter of sample.

The proton-decoupled ^{13}C spectra of VLDL, LDL, and HDL are shown in Fig. 1. Assignments of the resonances (Table 1) are based on comparisons with known ^{13}C chemical shifts of proteins (*4*), cholesteryl acetate (*9*), lecithin (*10*), and triglycerides (*11*). The most intense peaks in the spectra come from fatty acyl moieties, since these are present in all classes of phospholipids, cholesterol esters, and triglycerides.

The VLDL group contains a large proportion of triglycerides, and this is reflected in the ^{13}C spectrum, which resembles that of a triglyceride mixture (Fig. 1A). In contrast to the spectra of LDL and HDL, the VLDL spectrum shows the methine resonance of the glycerol moiety (peak 4 in Fig. 1A). Other than triglyceride resonances in the spectrum of VLDL, only the resonance of the choline trimethylammonium group (peak 6 in Fig. 1A) and a few weak cholesterol resonances (peaks 7, 8, 9, and 18 in Fig. 1A) are observed.

The spectra of LDL and HDL (Fig. 1, B and C, respectively) are qualitatively similar. The most prominent features can be assigned to fatty-acid, cholesterol, and choline resonances. The intense olefin peaks at 62.8 and 64.5 parts per million (ppm) in all three spectra are indicative of the high content of oleic and linoleic acids in human serum lipoproteins (*12*). Choline-containing phospholipids account for the bulk, 80 to 90 percent, of all lipoprotein phospholipids (*13*); consequently, the intensity of the $-N(CH_3)_3$ signal at 138.3 ppm serves as an indicator of the content of lipoprotein phospholipids. This resonance is particularly strong in the spectrum of HDL, reflecting the high phospholipid content of this lipoprotein class.

Several strong single-carbon resonances of the cholesterol moiety are observed in the spectra of LDL and HDL, which contain appreciable amounts of cholesterol, mostly in the form of esters. The molar ratio of phospholipids to cholesterol (free plus ester) is more than twice as high in HDL than in LDL. Thus, the intensity of the choline peak relative to that for several cholesterol markers is significantly greater in HDL than in LDL.

Although the HDL contain approximately equal amounts of protein and lipid on a weight basis, the sharp features of the ^{13}C NMR spectrum arise from the lipid components. A broad carbonyl signal (peak 2 in Fig. 1C) arises from the overlap of the many protein backbone amide carbonyls. The only other clearly visible protein feature is the carboxyl resonance of the glutamic acid side chains (peak 1 in Fig. 1C). The lack of prominent protein signals is due to two effects. First, lipids as a group contain fewer classes of carbon atoms than do the proteins. Second, a majority of protein carbons have short spin-spin relaxation times and consequently broad resonances (*14*). The narrow peaks in Fig. 1 arise from carbons with relatively long relaxation times ($\gtrsim$ 0.1 second). Long ^{13}C relaxation times are the result of short effective rotational correlation times (as in the case of carbons with fast internal rotations) or the lack of directly attached hydrogens (*3*). Protein contributions to the spectrum of HDL come primarily from the carbonyl carbons of the polypeptide backbone, while the ε carbon of lysine and the β carbon of leucine residues possibly contribute to the resonance at 153 ppm. Protein β and γ carbon atoms may contribute to the broad absorption envelope from 155 to 165 ppm but do not produce identifiable resonances. These conclusions are based partially on a ^{13}C spectrum of apoHDL (spectrum not shown).

The above considerations concerning ^{13}C line widths explain the fact that the only clearly observed cholesterol resonances are those of the nonprotonated carbons (C5, C10, and C13) and those of protonated carbons which are expected to have fast internal reorientation on the basis of ^{13}C relaxation studies on cholesteryl chloride (*3*), namely C24, C25, and the methyl carbons (C18, C19, C21, C26, and C27). Carbon 6 of cholesterol is the only "nonmobile" protonated carbon which yields a detectable broad resonance in the spectra of LDL and HDL (peaks 6 and 8 in Fig. 1, B and C, respectively). The line width is about an order of magnitude greater than that of unassociated cholesterol derivatives (*3*). This result is indicative of the restricted mobility of the cholesteryl moiety in lipoproteins.

These results establish that highly resolved ^{13}C NMR spectra of human serum lipoproteins can be obtained and assigned, and that qualitative aspects of these spectra can be correlated with the lipid composition of the particular sample. The observations strongly suggest that it will be possible to measure dynamic properties of these structures through the use of "partially relaxed" Fourier transform spectra (*3*) and to relate these properties to lipid composition.

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